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Association of Lipid biomarkers with drinking patterns and severity in alcoholic liver disease - A hospital- based cross- sectional study

Anbalagan Anithasri[1], Subramaniam Arulvijayavani[2]*, Poonguzhali Gopinath[3] & Annamalai Kumarasamy Varuni[4]

[1]Department of Biochemistry, Government Villupuram Medical College, Villupuram-605 601, Tamil Nadu, India
[2]Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Karaikal-609 602, Puducherry, India
[3]Department of Biochemistry, Government Tiruvannamalai Medical College, Tiruvannamalai-606 604, Tamil Nadu, India
[4]Government Villupuram Medical College, Villupuram-605 601, Tamil Nadu, India

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Alcoholic liver disease (ALD) is one of the leading causes of death due to cirrhosis. Dyslipidemia is a common finding in ALD and lipid parameters are shown to be associated with disease severity. However the effect of alcohol drinking pattern on lipid abnormalities is still unclear. Hence this cross sectional study was planned to estimate the serum lipid profile in ALD patients and to determine the association of lipid parameters with alcohol drinking pattern and the severity of liver disease.50 male patients with ALD and 50 age matched controls were enrolled. AUDIT score was used to assess their drinking pattern. Serum lipid profile and liver parameters were estimated and compared between the cases and controls. The patients were grouped based on severity into Child Pugh's group A, B and C and the study parameters were compared between the groups. All statistical analyses were done using SPSS v20.0. The mean total cholesterol level (126.98±45.06 *vs* 163.2±24.38 mg/dL), LDL and HDL cholesterol was significantly less in ALD compared to controls. Total cholesterol and LDL cholesterol levels were low in Child Pugh's Score C group. Mean AUDIT score was18 ± 4. Low Total cholesterol and LDL cholesterol levels correlate with disease severity and are markers of poor prognosis. Lipid parameters do not correlate with alcohol drinking patterns in ALD.

Keywords: Alcoholic Liver Disease, AUDIT score, Child Pugh's score, Dyslipidemia, Lipid Profile

Alcoholic liver disease (ALD) progresses from simple steatosis, steatohepatitis to cirrhosis and hepatocellular carcinoma. About 30 to 35 percent of alcoholics who present with fatty liver in due course progress to cirrhosis[1]. Nearly 50% of the deaths due to liver disease in the age group of 15 years and more is attributed to alcohol[2]. The per capita alcohol consumption of India has increased by 55% over the

change in the liver which progresses to acute or chronic hepatitis depending on various metabolic, lifestyle, ethnic and genetic factors. Alcohol consumption causes adipose tissue dysfunction that plays a key role in the pathogenesis of alcoholic liver disease[4]. Chronic alcohol intake increases the circulating Non esterified fatty acids that negatively modulates the adipokines resulting in a proinflammatory state. The cytokines released stimulate hepatic stellate cells leading to fibrosis and cirrhosis[5]. Both *in vitro* and *in vivo* animal studies have shown that treatment with ethanol causes over-expressing of SREBP-1 that induces hepatic lipogenesis and accumulation of cholesteryl esters and triglycerides[6].

Dyslipidemia in terms of decreased total cholesterol and high triglyceride (TG) and very low density lipoprotein (VLDL) levels has been [illegible]which correlated with sonographic and histopathological findings of Chronic liver Disease (CLD)[7]. Several studies have also found an inverse correlation of lipid parameters with severity in cirrhosis. Privitera *et al.* have shown that hypocholesterolemia is an independent predictor of survival in cirrhosis and triglycerides determines the severity of liver damage in alcoholic liver cirrhosis being the highest in Child Pugh Score B, whereas decreased HDL and increased LDL cholesterol play a pathogenic role in cirrhosis related complications[8]. In addition, the effect of alcohol on adiposity is variable depending on the

*Correspondence:
E-mail: arulvijayavani@gmail.com

type of alcohol, drinking patterns and individual metabolism of alcohol. Multiple controversial findings warrant an assessment of lipid profile in ALD indigenous to the population. Therefore the present study aims to estimate the serum lipid profile in alcoholic liver disease patients and its association with alcohol drinking patterns and the severity of liver disease.

Materials and Methods

The present study is a cross sectional study conducted in the Department of Biochemistry in collaboration with the Department of Internal Medicine at Government Villupuram Medical College as a part of ICMR STS (Indian Council of Medical Research Short Term Studentship) project. Ethical clearance was obtained from the Ethics committee (Human studies) of the institute. Informed consent was obtained from all participants included in the study. Male patients diagnosed as Alcoholic liver disease comprising fatty liver, acute hepatitis, decompensated liver disease or cirrhosis were included in the study while patients with liver diseases due to non alcoholic causes, patients with associated comorbities like Diabetes mellitus, Hypertension, Malnutrition, Renal failure, Malabsorption, Patients on chronic drug intake or parental nutrition were excluded[9-10]. Age matched males without history of alcohol consumption and comorbidities who attended the master health checkup clinic were selected as controls. A detailed history regarding the drinking pattern was recorded using AUDIT (Alcohol Use Disorder Identification Test) score[11-13]. A score of >8 indicates alcohol dependence. 5 mL of blood sample was drawn from the study subjects in fasting state for analysis of biochemical parameters by standard methods using automated chemistry analyser Beckmann Coulter AU480. Severity of the liver disease was assessed by Child- Pugh–Turcott score. The distribution of all biochemical parameters is expressed as Mean with S.D. The difference in the study parameters between cases and controls was done using independent student's *t*-test. The sub group analysis for comparison between the three Child Pugh's groups was done using ANOVA. Spearman correlation was used to assess the linear relationship between the study parameters. All statistical analyses were carried out at 5% level of significance using SPSSv20.0 software and *P* value less than 0.05 was considered to be statistically significant.

Results

The study included 50 male patients with Alcoholic Liver Disease and 50 age matched controls. The mean age of the cases was 42 years while that of controls was 39 years. Among the lipid parameters Total Cholesterol (TC), HDL Cholesterol (HDL-C) and LDL cholesterol (LDL-C) levels were significantly low in cases compared to controls while triglycerides and VLDL did not show any difference (Fig 1). The mean TC level of cases was 126.98±45.06 mg/dLwhich was significantly less than that of controls 163.2±24.38 mg/dL. The percentage of patients with a TC level of >200 mg/dL is 6% and those with a value of < 100 mg/dL is 32% and the remaining 62% had their cholesterol values in the range of 100 - 200 mg/dL. Similarly, HDL-C and LDL-C were significantly low in cases. The HDL was < 30 mg/dL in 16% of the cases while 68% of patients had LDL-C of <80 mg/dL. Though there was no significant difference in TG) between cases and controls, 8% of them had values >200 mg/dL (Table 1). The mean AUDIT score was 18±4. Out of 50 patients only 1 patient had an AUDIT score of less than 8. While the remaining 49 of them had an AUDIT score of >8 indicating alcohol dependence in almost all the cases. The Alcoholic Liver Disease patients were classified into mild, moderate and severe based on Child Pugh Score into Child Pugh's A, Child Pugh's B and Child Pugh 's C. TC and LDL-C were significantly low in Child Pugh's Score C group compared to groups A and B (Fig 2). TG, VLDL and HDL cholesterol did not show any significant difference (Table 2). Further TC and LDL levels had significant negative correlation with PT/INR and Child Pugh's score (Table 3). Total Bilirubin and Direct Bilirubin also had significant

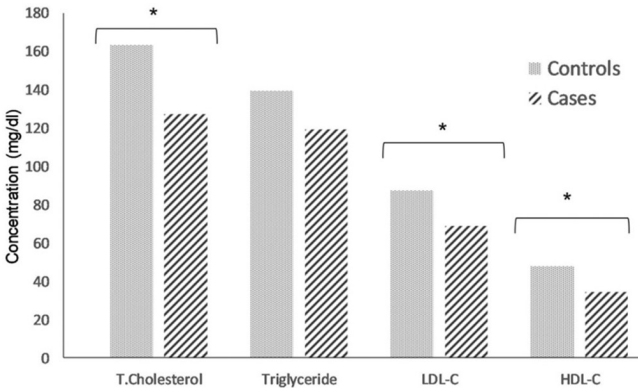

Fig. 1 — Comparison of lipid parameters between Cases and Controls. *denotes *P*- value statistically significant. Figure shows the difference in levels of TC, TG, LDL-C and HDL-C between ALD cases and controls

Table 1 — Comparison of study parameters between Cases and Controls

Parameters	Control (n=50)	Cases (n=50)	*P*- value
Age (Years)	39.06 ± 7.90	42.46 ± 10.47	0.07
Glucose(mg/dL)	80.63 ± 28.94	107.5± 45.87	0.001
Urea (mg/dL)	25.62 ± 9.0	20.1 ± 6.15	0.001
Creatinine (mg/dL)	0.84 ± 0.13	10.0 ± 0.38	<0.001
Total Bilirubin (mg/dL)	0.69 ± 0.18	2.24 ± 2.10	<0.001*
Direct Bilirubin (mg/dL)	0.21± 0.07	1.73± 1.83	<0.001*
Total Protein (g/dL)	7.23± 0.78	7.07 ± 0.58	0.28
Albumin (g/dL)	3.93 ± 0.64	3.24 ± 0.9	<0.001*
Total Cholesterol (mg/dL)	163.2 ± 24.38	126.98 ± 45.06	<0.001*
Triglycerides (mg/dL)	139.2± 24.38	119.24 ± 49.37	0.12
VLDL Cholesterol(mg/dL)	27.84 ± 4.88	23.35 ± 9.87	0.12
HDL Cholesterol (mg/dL)	47.96 ± 9.73	34.36 ± 5.40	<0.001*
LDL Cholesterol (mg/dL)	87.24 ± 20.40	68.77 ± 39.29	0.04*
PT/INR	0.96 ± 0.11	1.86 ± 0.62	<0.001*

HDL- High- density lipoprotein, VLDL- Very low density lipoprotein, LDL- Low- density lipoprotein, PT- Prothrombin time, INR-International normalised ratio *-*P* value statistically significant

Table 2 — Comparison of the parameters with mild, moderate, severe liver disease cases

Parameters	Child Pugh A (n=13)	Child Pugh B (n=23)	Child Pugh C (n=14)	*P*- value
Age (Years)	43.46 ± 12.14	45.39 ± 8.16	36.71± 10.63	0.043*
Glucose(mg/dL)	93.38 ± 29.03	118.82 ± 58.13	102.00 ± 31.20	0.25
Urea (mg/dL)	20.08 ± 4.23	19.74 ± 8.11	20.71± 3.77	0.90
Creatinine (mg/dL)	1.17 ± 0.35	0.98 ± 0.37	1.11± 0.43	0.33
Total Bilirubin (mg/dL)	1.14 ± 0.84	1.34 ± 1.12	4.72 ± 2.07	<0.001*
Direct Bilirubin (mg/dL)	0.85 ± 0.87	0.94 ± 1.07	3.76 ± 1.89	<0.001*
Total Protein (g/dL)	7.01 ± 0.65	7.16 ± 0.52	6.99 ± 0.62	0.63
Albumin (g/dL)	3.62 ± 0.90	2.98 ± 0.81	3.37 ± 0.93	0.077
Total Cholesterol (mg/dL)	144.46 ± 28.63	134.48 ± 56.97	98.42 ±10.95	0.013*
Triglyceride (mg/dL)	121.77 ± 42.07	122.96± 57.13	110.79 ± 43.85	0.76
VLDLCholesterol (mg/dL)	24.35 ± 8.41	24.59 ± 11.43	22.16 ± 8.77	0.76
HDL Cholesterol (mg/dL)	36.54 ± 5.69	33.47 ± 4.04	33.79 ± 6.77	0.24
LDL Cholesterol (mg/dL)	83.57 ± 23.90	76.41± 49.05	42.49 ± 12.44	0.009*
PT/INR	1.32 ± 0.22	1.73 ± 0.43	2.58 ±0.49	<0.001*

TG - Triglyceride, HDL- High density lipoprotein, VLDL- Very low density lipoprotein, LDL- Low density lipoprotein, PT- Prothrombin time, INR-Internatcorional normalised ratio*-*P* value statistically significant

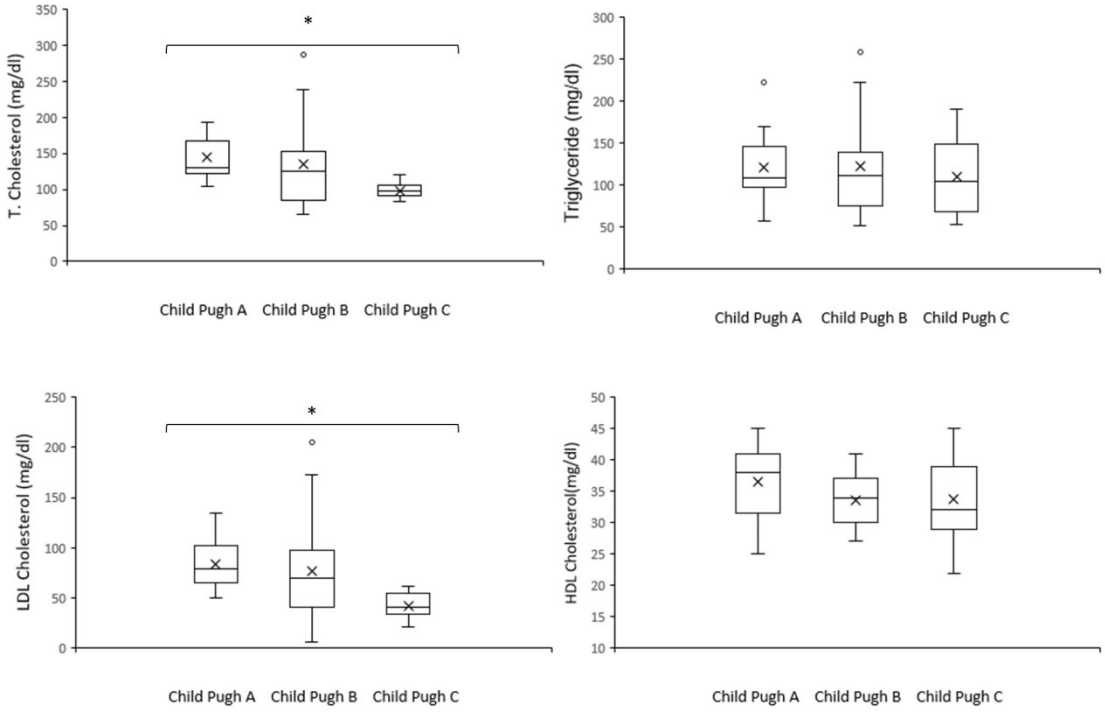

Fig. 2 — Comparison of lipid parameters between Child Pugh's A, B and C groups. *denotes *P*- value statistically significant. Figure shows the difference in levels of TC, TG, LDL-C and HDL-C between Child Pugh's A, B and C groups

Table 3 — Correlation of the parameters with Child Pugh's Score and PT/INR value

Parameters	Child Pugh's Score		PT/INR value	
	r value	*P*- value	r value	*P*- value
T. Cholesterol	–0.53	0.00*	–0.47	0.001*
LDL-C	–0.53	0.00*	–0.509	0.001*

LDL-C low density lipoprotein cholesterol, *- *P* value statistically significant

positive relation with PT/INR. However the lipid parameters did not show significant correlation with AUDIT score while Total Bilirubin had a significant positive correlation with AUDIT score (r =0.296, *P* =0.03).

Discussion

ALD is associated with derangements in lipid and lipoprotein metabolism due to the toxic effects of alcohol on peripheral and hepatic lipid metabolism. There is increased triglyceride synthesis, decreased esterification of cholesterol as well as abnormalities in structure and composition of lipoproteins in Alcoholic cirrhosis and the severity of cirrhosis is inversely related to Body mass Index[12].The present study shows a decrease in the mean TC and LDL cholesterol levels. Alcohol induces PNPLA3 gene that predisposes to increased lipogenesis and facilitates fibrosis progression in ALD. Alcohol exposure increases the micro RNAs that causes upregulation of Lipopolysaccharide (LPS) signalling in Kupfer cells that releases pro inflammatory cytokines like TNF-α and IL-6. This leads to progression of hepatitis and steatohepatitis [13].

As evident in other studies by Peng *et al.* and Khan *et al.* the mean cholesterol level is less than the normal value suggesting that the metabolism of cholesterol in liver is being severely affected. The triglyceride levels were normal whereas the cholesterol levels were low in ALD patient since the apoprotein synthesis were modified in liver disease which led to lower cholesterol and normal triglyceride levels. Peng *et al.* documented marked decrease in total cholesterol levels in 15% of chronic liver disease cases, while 82.5% of them had a low to normal range of cholesterol and 63.13% had low to normal triglycerides[14].In the study by Khan *et al.* in CLD patients they found high TG and low LDL-C

levels in cirrhotics compared to healthy controls while there was no significant difference in TC and HDL levels[15]. However the present study shows a significant negative correlation of TC and LDL-C with severity of liver disease. As TC and LDL-C were very low in Child Pugh's C group compared to mild and moderate groups this makes low TC and LDL a marker of poor prognosis in alcoholic cirrhosis signifying extremely decompensated state of hepatocytes.

A study conducted by Arain *et al.* aimed to assess the degree of alteration of serum lipid profile in Hepatitis B virus cirrhotic patients found that the serum triglyceride, total cholesterol, HDL and LDL cholesterol was significantly low in HBV cirrhosis[16]. A study conducted by Phukan *et al.* aimed to assess the degree of alteration of serum lipid profile in alcoholic cirrhotic patients and also to detect its relationship with alcohol consumption pattern. The serum total cholesterol, HDL and LDL cholesterol was significantly low in cirrhotics while, the serum triglyceride levels were increased. However they did not find any correlation of lipid parameters with amount and duration of alcohol consumption. And they have suggested routine screening of alcoholic cirrhotic patients for lipid profile abnormality[17]. Another finding from this study is that the AUDIT score correlated positively with bilirubin levels proving that alcohol dependence causes increased hepatocyte damage leading to cirrhosis. This is in par with the findings of Adak *et al.* where they have found correlation of hyperbilirubinemia and hypocholesterolemia with alcohol intake[18]. This study is the first of its kind to correlate AUDIT score with lipid profile in our population. It is noteworthy that only one patient of our study population had AUDIT score less than 8 indicating the impact of alcohol drinking pattern on the severity of liver disease. So it is suggested that either abstinence or change in alcohol drinking pattern should be a part of effective management and prevention of ALD.

The results of the study conducted by Boemeke*et al.* revealed that hypocholesterolemia is associated with hepatic progression of cirrhosis in alcoholic and viral cirrhosis[19].In a retrospective study of newly diagnosed cirrhosis patients, hypercholesterolemia was associated with decreased mortality in Child-Pugh category A and B[20]. The strength of the study is the exclusive selection of ALD cases evaluating alcohol dependence and its correlation with dyslipidemia. Unlike majority of previous studies about dyslipidemia in chronic liver disease which included the liver disease of viral and non-viral etiology, the present study is centred on the alcoholic cause of liver disease which is avoidable and preventable.

Conclusion

Total cholesterol, HDL, and LDL cholesterol levels are decreased in ALD patients. Decreased total cholesterol and LDL cholesterol levels correlate with disease severity in ALD and are markers of poor prognosis. However, lipid parameters do not correlate with alcohol drinking patterns. Dyslipidemia is a commonly associated clinical finding in liver disease that is often overlooked. Early and adequate management of lipid derangements in ALD must be considered.

Conflict of interest

All authors declare no conflict of interest.

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